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THE MORPHOLOGY OF ARAUCARIA BRASILIENSIS
III. FERTILIZATION, THE EMBRYO, AND THE SEED

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(WITH PLATES I–III)

Since the publication of the author's account of the male (**3a**) and female (**3b**) gametophytes of *Araucaria*, certain additional facts have been observed. They will be recorded here by way of preface to the following account of the embryo and seed.

In the paper on the male gametophyte the pollen was described as lodging on the ovuliferous scale and then growing over the surface to the micropyle, and a figure was given showing a number of pollen tubes. These pollen tubes were shown pursuing a more or less direct course to the micropyle. It was stated that they sometimes crossed from the upper surface of the scale on which they had germinated to the under surface of the scale above. Since that was written EAMES has described the behavior of the pollen tubes of *Agathis* (**8**). The very interesting behavior of these tubes led me to re-examine those of *Araucaria*. It was found that while they do not, apparently, penetrate the tissues of scale and cone axis in the remarkable fashion characteristic of the sister genus, they do, nevertheless, branch much more profusely than had been supposed. It is often possible to separate a branching tube from the adjacent scales without breaking many of these branches. From this it appears that they are comparatively superficial. There is usually one main branch of the tube that goes more or less directly to the nucellus. From this numerous,

much smaller branches are given off. These branches run in all directions over the surfaces of the adjacent scales, so that when they are separated the mesh of tubes sometimes appears like a fine spider web. Numerous nuclei were distributed throughout the tubes, sometimes singly and sometimes in groups of a half-dozen or more. Aside from the body cell they all look much alike, so that it was not possible to identify the stalk and tube nuclei. The tube branches appear to grow independently of the tube nucleus or of any other nuclei. Branches were sometimes found in which no nuclei could be found at all. This was true of those of considerable length, as well as those that were just beginning to form. No cross-walls were observed in any part of the main tube or its branches. The main branch after entering the nucellus sometimes branches again (fig. 1). These branchings are very much less frequent than those outside. The available evidence indicates that only the one branch enters the nucellus.

In the majority of the tubes examined the body cell remains in the extra-nucellar part of the tube until after its division. Sperms were often observed in the tubes at the extreme tip of the nucellus (fig. 5), while very few body cells were observed within the nucellus. The mitosis of the body cell nucleus was not observed. Fig. 2 shows what appears to be the spindle lying between the two nuclei of a body cell. The nuclei are both in the resting condition and the spindle is surrounded by a delicate membrane. This membrane and its continued persistence after the daughter nuclei have reformed may be urged against this structure being really a spindle. It strongly suggests the remains of the nuclear membrane and thus suggests an intranuclear spindle. I have observed no other structures with which it could be identified, unless there may be some connection with the blepharoplast-like body referred to in a later paragraph as occurring in the cytoplasm of the male cells and the two-celled proembryo.

After the division of the body cell nucleus the two daughter nuclei do not usually separate for some time. In some cases they even enter the archegonium still closely associated. The division of the cytoplasm of the body cell follows considerably later. In some cases no distinct division has occurrred when the two male

cells have reached the archegonium. The body cell is commonly surrounded by a very faint membrane (figs. 3 and 6). In some cases this membrane is either absent or so faintly marked as to be exceedingly difficult of observation. The line of demarcation between the two male cells is in most cases not well defined. There does not appear to be a distinct membrane even when the two masses of cytoplasm are seen to be clearly separable. These male cells are not organized in definite cells as they are in cycads and *Ginkgo*.

At the time of division the two nuclei are of approximately equal size. They may both develop in similar fashion and share the cytoplasm equally (figs. 3 and 4), or one of them may degenerate even after the partial division of the cytoplasm. Fig. 6 shows a male cell or sperm clearly bounded by a limiting membrane and with the nucleus at one extremity. Across it may be seen the faint line of demarcation between the two original cells. One nucleus has degenerated and only the faintest trace of it can be distinguished in the tail opposite the functional nucleus. This degeneration is probably fairly common, for one often finds unusually large male cells in tubes in which no trace of the other one can be found. It is possible, of course, that it has retreated up the tube and into the extra-nucellar portion and so escaped observation. I incline to the opinion, however, that it has either degenerated or slipped out of the cytoplasm and become so reduced in size as to be indistinguishable from the prothallial and other nuclei.

In a previous paper (3a) it was stated that these male cells might possibly be motile. Since then many tubes have been dissected out in sugar solution in the attempt to prove this. The results have been entirely negative. They are apparently amoeboid (as for that matter are the male cells of some angiosperms), but there are no structural evidences of locomotor organs of any sort, nor were any rapid movements of any kind observed. Fig. 5 shows a binucleate body cell rounding a sharp corner of the pollen tube at the point where it turns back into the nucellus after having crept along over the surface of the scale. The distortion in shape is not in any way due to crowding, for there was abundant room for

it to have passed the corner without having altered its shape in the least. It was evidently creeping along the convex side of the tube and was killed just as it was rounding the corner. That the male cells actively change position and shape on their own initiative is shown also by the manner of entering the archegonium, to which attention will be called in a succeeding paragraph.

In the account of the archegonium already published (3b), it was stated that no ventral canal cells or nuclei had so far been observed, but that it was unlikely that they were not formed. Archegonia have since been observed in which such a ventral canal nucleus (figs. 7 and 8) had been cut off. There still remains some doubt whether this nucleus is regularly cut off. All the cases observed were in gametophytes some of whose archegonia had already been fertilized. I have not yet found a gametophyte with archegonia unfertilized where ventral canal nuclei were present. Two explanations are tenable. Either this division is delayed almost up to the time of fertilization, and the canal nucleus degenerates very quickly so as to leave no trace of itself in fertilized archegonia, or it does not occur normally, but only in those archegonia in which fertilization has been delayed beyond the usual time. Though the latter appears the less probable supposition on general grounds, the evidence available is more in accord with it. In one case (fig. 8) two small nuclei were present. The egg nucleus in this case appears small. What would ordinarily be taken for the ventral canal nucleus is larger than in fig. 7. In the majority of cases the cytoplasm of the egg in which ventral canal nuclei were found appears to be undergoing degeneration. It clumps together and has very indefinite structure. It cuts with difficulty. In some archegonia, of normal appearance in other respects, there was present a zone of fibrillar cytoplasm surrounding the very large nucleus. These archegonia were also invariably found in gametophytes with one or more that had already been fertilized. EAMES (8) reports the regular formation of the ventral canal nucleus in *Agathis* immediately before fertilization, and its rapid degeneration. COULTER and LAND (6) were unable to demonstrate its formation in *Torreya*. The expectation, therefore, is strongly in favor of its being formed normally before fertilization.

I have not been able to decide from the evidence at hand which is the more probable hypothesis.

In preparation for fertilization the egg nucleus enlarges considerably. Fibrillar cytoplasm is not uncommon in mature eggs, and sometimes, as pointed out in a preceding paragraph, forms an inclosing sheath when for any reason fertilization is delayed. The fibers of these sheaths run tangentially to the nucleus and remind one somewhat of the development of spindle fibers. They are much more abundant than is usual in the development of a multipolar spindle, and furthermore there is no reason to suppose that these mature egg nuclei are about to divide, unless for the formation of the ventral canal cell nucleus. I have seen no evidence whatever of their actually developing a spindle. Moreover, the archegonia appear to have passed their maturity already. Yet, in view of the uncertainty of the ventral canal nucleus being cut off, this possibility cannot be entirely excluded.

The nuclear membrane is well developed and incloses a relatively small mass of chromatin distributed on a fine linin network. The whole is immersed in a large volume of nuclear sap. Fig. 9 shows these facts very clearly, except that the wrinkled condition of the nuclear membrane doubtless indicates that considerable shrinkage in volume has occurred through the application of reagents. The chromatin is distributed in more or less definite strands of beadlike masses on the very delicate linin. The total number of these chromatin masses in a nucleus is very large. The total mass is also surprisingly large when one considers its volume in the fusion nucleus and in the first two nuclei of the proembryo.

Fertilization

The archegonia are mature and ready for fertilization in California about the last week of March or the first week of April. At this time the egg nucleus is usually situated a little above the middle of the archegonium. The egg cytoplasm has almost completely filled the archegonium. The vacuoles that were so conspicuous in the earlier stages of development have all disappeared. Neither starch nor other form of stored food seems to be present in the cytoplasm. As will be shown presently, the cytoplasm

itself is used up in the growth of the proembryo. The neck is composed of about 12 cells arranged in a single tier (usually). The nuclei lie at the larger peripheral end of the cells. In the pointed central end there is little cytoplasm. Often there appears to be a slight opening among the neck cells, as if in anticipation of the entrance of the sperm.

The course of the pollen tube after reaching the female gametophyte is not always direct. It frequently wanders along between the megaspore membrane and the gametophyte, eroding the latter more or less, before turning down into an archegonium. Archegonia that are apparently mature and ready for fertilization may be passed and the male cells delivered to archegonia some distance farther away from the point of entry of the pollen tube. Very commonly the archegonial chamber above each archegonium becomes overgrown, so that the tube must force its way down to the neck. Some cells are destroyed in its approach, but the way is more often prepared by the thrusting aside of the intruding cells and the consequent opening up of the previously existing passage.

When the tube reaches the archegonium its tip is thrust down into the immediate neighborhood of the neck cells. The tip is then ruptured and the male cells crowd through the narrow passage of the neck. Sometimes both male cells enter and more frequently only one can be found. The archegonia are often so crowded with cytoplasm that the entrance of the male cell causes the extrusion of some of it through the neck (fig. 10). The entry appears to be violent, for the egg nucleus is commonly driven to the bottom of the archegonium and may even be driven through the bottom. The violent displacement of the egg nucleus shows very clearly that the male cells move with considerable force, while the extrusion of the cytoplasm seems to prove that they are not forced in by the tube, as has been said to be the case in certain other gymnosperms. It seems clear from the facts just stated that these male cells are actively motile, although it is almost certain that they have no cilia or other organs of locomotion. Their large size as well as their vigor of movement makes them conspicuous among Coniferales. Such size and movement are matched only among the cycads and *Ginkgo*.

After the contact of egg nucleus and male cell the cytoplasm of the latter gradually envelops them both. Fig. 11 shows a case where both are completely enveloped in a common cytoplasm even before the nuclei have begun to fuse. In figs. 12 and 13 each nucleus is accompanied by a distinct cytoplasmic sheath even though fusion is far advanced. The egg cytoplasm is generally much disturbed by the passage of the male cell through it and does not ordinarily recover its structure. The cytoplasmic sheath around the fusion nucleus, on the contrary, continues to grow rapidly, apparently at the expense of the general cytoplasm of the egg. With the growth of the proembryo the egg cytoplasm gradually disappears, until there is commonly very little of it when the walls are formed in the former. In some cases (upper right of fig. 32) the mass of dense cytoplasm surrounding the proembryonic nuclei becomes delimited from the egg cytoplasm by a distinct membrane. So far as I have observed, this membrane has nothing whatever to do with the walls of the upper tier of the proembryo, which form later and entirely within the limits of this membrane. Fig. 31 shows a small portion of this membrane in the upper part of the figure, just above the largest cell shown. The left-hand cell shows distinctly the beginning of the formation of the walls. The wall is less clearly shown in the other cells, though the plasmatic membrane around their dense cytoplasm shows clearly where it will form. This membrane does not always form, and I am unable to see any significance that may be attached to it. The cap of cytoplasm spoken of by EAMES (8) as occurring above the upper tier of nuclei in *Agathis* is not ordinarily present in *Araucaria*, though fig. 27 shows a band that might be interpreted as such a structure. This figure also shows very clearly the previous delimiting membrane over the upper surface, in fact extending over that part of the embryo formed by the previously mentioned cytoplasmic cap. No distinct membrane is to be seen in figs. 25, 26, and 30, even though there is sharp distinction between the egg cytoplasm and that of the proembryo. A membrane entirely around the proembryo is shown in figs. 28 and 29. The younger stages apparently do not possess membranes of any sort.

When the male cell enters the archegonium it consists of dense cytoplasm inclosing a solid and compact nucleus. It rapidly enlarges before actual contact with the egg nucleus. At the time of contact there is much more nuclear sap and apparently rather less chromatin. The condition of the nucleus before contact is fairly well shown in fig. 16, showing (above the 2-nucleate proembryo) the second male cell. This male cell is beginning to degenerate and is in consequence somewhat more dense and homogeneous than the functional one. It should be compared with figs. 11–15. The egg nucleus contracts instead of expanding. It also appears to lose much of its chromatin. A comparison of figs. 11–15 with fig. 7, all photographed at the same magnification, will make this point clear. At the time of fusion the two nuclei are not very different in size. It is rather difficult to obtain an accurate notion of their comparative sizes because of the markedly different shapes. The egg nucleus usually remains round (figs. 11–14), while the sperm nucleus becomes concave on the side pressed against the egg nucleus. In consequence, it spreads out laterally so as to cover a third to a half of the surface of the egg nucleus. Its change of shape is accompanied by a loss of nuclear sap, but probably not of chromatin. The two nuclei remain in contact for some time, as shown by the frequency of this stage in my preparations as compared with some other stages. The manner of fusion is shown in fig. 15. A perforation between the two nuclei is formed and the gap stretches until the contents of the two nuclei are contained in a common cavity. The nuclear membrane of the fusion nucleus thus consists of parts of both the sperm and egg nuclear membranes.

The chromatin of the two nuclei enters the fusion nucleus in the form of coarse or fine nets. Fig. 15 shows the chromatin as fine granules distributed evenly throughout the two nuclei. Fig. 13 shows granules arranged in series that might easily correspond to individual chromosomes. Other preparations show various gradations between these two extremes. Whether the two masses remain separate, as is said to be true in *Pinus* (**5, 9a, 14, 15**) and some other gymnosperms, could not be determined from the available material. No preparation showing the fusion nucleus after

complete fusion and before complete division was secured. I have been forced to the conclusion that this stage must be of very brief duration.

The relative position of the two sexual nuclei varies somewhat in different archegonia. The male cell probably comes in contact with the upper side of the egg nucleus. In many cases this relative position is shifted through the violence of the impact, so that the male cell may lie more or less to one side or even far around toward the bottom (fig. 13).

The second male cell sometimes enters along with the functional one. I have seen no indications of its functioning in the manner reported for *Agathis* (8), or in any other manner. When it enters it soon degenerates (fig. 16). I have seen no evidence that it ever divides, as has been reported for some other conifers (9a, 9b).

Attention has been called to certain peculiar bodies in the cytoplasm around the fusing sexual nuclei and sometimes in that of the 2-nucleate proembryo. Fig. 15 shows two of these bodies. The one to the left may possibly be a disintegrating vegetative nucleus from the pollen tube, though I do not think so. The one lying in the cytoplasm between the nuclei certainly is not of this nature. They are not found in every cell, but occur frequently enough to be legitimate objects of curiosity. They suggest the blepharoplasts of the cycads. When they were first observed a diligent search was instituted immediately for similar structures in the body cell and the male cells before they enter the archegonium. The results were entirely negative. Fig. 17 shows one in the second male cell within the archegonium. The division of the fusion nucleus has not been observed, and it is possible that they may function here as blepharoplast-like or centrosome-like bodies.

Proembryo

The division of the fusion nucleus probably follows soon after the complete union of the egg and sperm nuclei. The resulting nuclei may lie one above the other, side by side, or in an oblique plane (figs. 17–19). They vary considerably in size, as is evident from a comparison of figs. 18 and 19. Before the next division there is a moderate increase of cytoplasm. The two nuclei

probably divide simultaneously, since no 3-celled proembryos were found. No mitoses in the proembryo have been observed, nor any trace of evidence that the nuclei divide amitotically. I have already, in former papers (**3a, 3b**), called attention to the very curious fact that almost no mitoses in the critical stages of development of *Araucaria* have been observed. It is a very curious and puzzling fact, not to say a very annoying one. SAXTON has recently called attention to a similar state of affairs in another southern hemisphere form, *Actinostrobus pyramidalis* (**18a**).

The four free nuclei may occupy almost any position with reference to one another. It has already been mentioned that the position of the fusion nucleus appears to depend on how much it is displaced through the violence of the contact between egg and sperm. It may lie near the middle of the archegonium, as it does in *Agathis* (**8**), or more generally near the bottom. The succeeding divisions take place wherever the fusion nucleus has been left. This same displacement would probably tend to conceal any polarity that the fertilized egg might possess. The commonest appearances of the proembryos are shown in figs. 20 and 21. Sometimes the four nuclei may all lie at the bottom of the proembryo, as in figs. 23 and 25. The subsequent divisions do not appear to follow any definite order nor are they simultaneous. Whether the 4 nuclei were tetrahedrally placed (figs. 20, 21), placed in a single vertical plane (fig. 23), or in a curved line around the bottom (fig. 23), or in any other position, seems not to affect the ultimate result. Irregular division continues for two weeks or more before the final arrangement of the cells in tiers. The number of cells or free nuclei at this time varies considerably. No counts of less than 32 nor more than 45 were obtained from an examination of a considerable number of embryos of about this stage. The number of proembryos showing the beginning of wall formation was so small that it cannot be certainly said that some of those with 32 free nuclei might not have had more at the time of wall formation. Many of these seemed as large and as definitely arranged as the ones with a greater number of nuclei. It seems to me, therefore, that the number of nuclei at the time of wall formation is probably variable.

After or just about the time of the cessation of free nuclear division the nuclei arrange themselves as shown in fig. 30, which is a median vertical section. It will be seen that there is a central group of nuclei arranged more or less regularly in two tiers, surrounded by a complete jacket of peripheral nuclei. These peripheral nuclei are usually more numerous on the lower side than on the upper. Even before walls are formed the lower nuclei sometimes begin to elongate, foreshadowing the formation of the cap. Fig. 32 shows a proembryo in which walls are forming about the lower nuclei, which are already set off in definite cells. The walls appear to form first in that part of the embryo which first begins elongating. In fig. 32 the lower cells formed first and began elongating while there is yet no indication of the future cells in the upper portion of the proembryo. Precisely the opposite state of affairs is shown in fig. 31, where the upper cells are elongating and forming walls while the lower cells are just forming but have not begun elongation and have only faint traces of walls around some of them.

After the complete establishment of walled cells the elongation which had already begun continues simultaneously in both the upper and lower cells. It is only after this elongation that one may properly speak of tiers, for, as already pointed out, the cells are arranged concentrically rather than in layers. Few or no divisions occur in the terminal group of cells, destined to form the cap, during this preliminary elongation, and none at all subsequently. There is a considerable increase in the upper group. They divide longitudinally, so that there are ordinarily about twice as many cells in the young suspensors as there were in the group of cells from which they were developed. The number shown in cross-section varies somewhat, but is usually not far from 20. As the suspensor cells elongate, their upper ends are thrust backward and upward (that is, in the direction of least resistance) until they encounter the firm top of the archegonium. Their upper ends ordinarily become swollen during elongation, so as to stretch the upper part of the archegonium (fig. 33). Incidentally this figure also shows that the neck is not ruptured by the entrance of the male cells and is not torn away from its mooring to the

upper part of the jacket as EAMES has shown to be the case in *Agathis* (8).

The cap is completely organized by the time the elongating suspensors have reached the neck of the archegonium. Owing to the greater elongation of the central cells of the cap than of those in each successive circle back of it, the cap has a much more pointed appearance than the proembryo at first exhibited. Fig. 35 shows a mature cap. It exhibits very clearly the relations of the component cells. This figure also brings out very clearly the fact that the tiered appearance of the embryo is more apparent than real, for the cap is really formed of all the cells of the peripheral layer below the suspensor. The embryonic group lies in a cup-shaped depression in the top.

The embryonic group of cells consists of a hemispherical or globular mass of small cells. There are usually 20–24 cells in the hemispheres (fig. 35), but there may be as many as 30 or even more in the globular masses. The number contained in the proembryo remains unchanged from the time they are set off and walled in until after the development and elongation of the primary suspensors.

After the organization of the walled proembryo and its preliminary development of the cap and an anchorage in the top of the archegonium, the suspensor cells begin a rapid elongation, accompanied by transverse division. This pressure of elongation maintains a firm contact of the cap with the cells in front of it. The suspensors at first thrust straight downward toward the center of the endosperm. This stage of development is probably accomplished quite rapidly, for most preparations show either free-nuclear proembryos or long, coiled suspensors (fig. 37). Usually more than one embryo starts development, about three of which start near enough at the same time to make the race for position in the center of the endosperm (fig. 36) a spirited one. When they have reached the center, the competitors coil around one another in the struggle for supremacy. One finally emerges below (fig. 37) —the victor. The others ordinarily perish without further development, though not a few cases have been seen where a second embryo had reached some such degree of development as that shown in

fig. 40 or 41. I have found no seed with a second embryo large enough to be seen with the naked eye.

Ever since STRASBURGER'S account (**23**) of the cap of the proembryo of *Araucaria brasiliensis*, it has excited comment on its apparent specialization. It has been spoken of as a protecting cap (**7, 20, 23**), but no evidence has been adduced to show that protection is at all necessary. The caps are not made of specially strong cells, nor do they show any effects of abrasion, which might reasonably be expected if they were of use as a protection. Neither do the cells of the endosperm surrounding the caps appear to have been crushed and thrust out of the way. It seems much more likely that the cells of the cap secrete a digestive enzyme. An inspection of figs. 34–37 will make this evident. Very few cells of the endosperm show any distortion from crushing, while practically all of them show the action of some corrosive agent on their contents or even in some cases on the walls themselves. I have seen no evidence, however, for thinking that the secretion of enzymes is limited exclusively to the cap. In fig. 34 it will be seen that the region of greatest cell destruction is around the embryonic region and not directly in front of the advancing tip. It is clear from fig. 37 that the cavity in which the proembryos lie continues to enlarge around the suspensors long after the cap has passed by. To put the argument in another way, the cavity should be cylindrical if solution occurs only around the cap, whereas the cavity is actually shaped like a wide-mouthed cone, showing that solution has gone on all over its surface and not merely at the apex of the cone. It is, of course, possible that this might be true and still all of the enzymes be formed in the cap, but excreted in such abundance that they fill the entire cavity with a solution of equal strength. I suspect that the matter comes to about this. The cap looks like a highly specialized structure and should in consequence have a specialized function. The proof that it does actually have a special function has not yet been adduced.

Sometime in June or July the primary suspensors have reached their limit of elongation. Then begins the third and final stage of development of the proembryo. The activities of this stage are limited to the embryonic group of cells. A rapid multiplication

of its cells is the first step. Fig. 38 shows an early stage in this growth. As soon as it begins the cap cells begin to disintegrate and are soon crushed (figs. 38 and 39). At first all the cells divide with equal rapidity. Very soon the upper cells show a tendency to enlarge, and more especially to elongate, while the lower ones continue division unabated. An early stage of this phase of development is shown in fig. 39 and a later one in fig. 40. The proembryo now consists of two regions: (1) The very actively dividing cells at the tip constitute a large apical meristem, and (2) the cells behind the meristem gradually cease division and elongate so as to produce a massive secondary suspensor which pushes the proembryo still farther down into the endosperm. After a time (a month or so) the proembryo consists of a massive suspensor and a large cylindrical body of meristematic tissue. The activity of the apical meristem practically ceases.

Embryo

Three new meristems are now developed. The first of these is picked out where the suspensor joins the main body and is to form the growing point of the hypocotyl. The other two form either side of the original growing point and quickly develop the two cotyledons. The remains of the primary meristem constitute the meristem of the stem apex, which continues dormant until some time after germination of the seed. Fig. 42 shows a longitudinal section of such an embryo some time in early September. All the regions of the embryo are now in course of development. Stem and root apices, cotyledons, and vascular tissues are clearly in evidence. These regions continue growth for two months or more before the seeds have reached the shedding stage. Growth in this period is largely confined to the cotyledons, which become very large in comparison with the hypocotyl.

The distribution of the vascular tissues in the embryo is shown in figs. 42–44. The cotyledons are traversed by 7 vascular bundles. Each of these can be traced backward to its separate union with the vascular cylinder of the hypocotyl. In the latter the procambium strands form a hollow cylinder. Just below the origin of the

bundles of the cotyledons the vascular cylinder is more or less quadrangular. The longer axis lies in the plane of the cotyledons. The other two sides are more weakly developed and bend in slightly toward the stem apex. The vascular cylinder rounds up gradually as it extends toward the root.

Resin canals are abundant in the cortex of the embryo, but do not occur in the pith or wood. There is a fairly regular circle of them three or four cells beneath the epidermis. Another definite circle occurs just outside the procambium strands. There is no definite boundary between stele and cortex, and so I am somewhat uncertain whether this ring of ducts should be attributed to the cortex or to the pericycle. Some authors (**20**) apparently speak of all the outer portion of the embryo as pericycle. I can see no good reason for this usage. The tissue is all alike at first. Then the procambium strands arise in the central region, inclosing a region of parenchymatous cells and are in their turn surrounded by a similar parenchymatous region. There is a pretty regular correspondence in number and position between the procambium strands and the resin ducts. Between the inner and outer circles there are numerous other less regularly disposed ducts. The preceding facts are shown in fig. 43, though not so clearly as I should have liked. Resin ducts occur in the cotyledons also. In the base they accompany the vascular bundles and are just below the epidermis of the outer face of the cotyledon, but not on the inner side of the bundles. Farther out toward the tips the outer ring extends completely around beneath the epidermis, in much the same way as in the hypocotyl. Resin ducts do not appear to extend upward in the embryonic mass from which the final embryo is differentiated beyond the dark line shown in the upper part of fig. 42.

In the subsequent growth of the embryo the hypocotyl changes very little, while the cotyledons elongate enormously. At the time the seeds drop from the cone axis (late November to January), the embryo is about 3 cm. long, of which the hypocotyl forms about 5–6 mm. At this time the embryo is quite straight and extends to within about 1 cm. of the tip of the endosperm. The hypocotyl is crowded closely into the apex of the seed.

After the fall of the seed the embryo continues to grow unless it becomes excessively dry. Fig. 48 shows a longitudinal section of a seed that had been stored in a tin box in my laboratory for a year and a half at least, and possibly two and a half years. The seed has continued the development of the embryo in much the same manner that would have occurred if it had been planted, except that development has gone on at a much reduced rate. Many other seeds in the same box put out roots 3–4 inches long within 6–8 months. When planted the hypocotyl emerges in the spring following the winter in which the seeds were shed. As these seeds are often shed in California before the rainy season has begun, it is evident that this intraseminal development is a means of enabling them to make the most of the growing season when it does come. It is not unlikely that in their native habitat this habit is equally useful. The seeds that did not continue growth appeared not to have done so on account of the attacks of a fungus that reduces the endosperm to a fine white dry powder. The embryos become yellow, shrunken, and waxy. The proportion of seeds failing to sprout was much the same in the box on my shelf as when they were properly planted. In fact, complete burial seems to be unfavorable to successful germination.

Endosperm and seed

During the development of the embryo important changes occur in the female gametophyte. The young gametophyte consists of comparatively large, very thin-walled cells with exceedingly scanty contents. They are multinucleate at the time of fertilization. By the time the proembryo has used up the food supplies of the archegonia and has begun to push down into the gametophyte, the cells immediately below have increased their cytoplasm markedly (figs. 34, 36, 37). The nuclei also increase in number. As the embryo advances the zone of food formation and storage precedes it. In the center of the endosperm there is left a narrow space more or less free from food storage. Toward this latter the embryos direct their course. The region of growth and storage of foods is below the archegonia. The upper region shrivels up and is crowded back into the apex of the developing seed. The

lower part of the gametophyte enlarges many fold. This growth is due in part to cell multiplication, but more largely to the increase in size of the already existing cells. As they enlarge they form and store up starch and multiply their nuclei up to 4 or 5 in almost every cell, and in some of them to twice these numbers. Fig. 46 shows nearly all of a single cell taken from about the middle of the endosperm. The light lines near the border mark the position of the delicate cell walls, which do not show in the photograph. The large oval bodies are starch grains, and the small round ones are proteids. Many of the cells are so crowded with food as to make photographs difficult. The proteid granules appear much later than the starch grains. They are not distinguishable optically much before the stage of the embryo shown in fig. 42. They never become so large or so numerous as the starch grains. Much the larger part of the growth of the gametophyte occurs during this period of food formation and storage subsequent to fertilization. At this period it is not more than 5–6 mm. in length, while at maturity it is about 4 cm. long and 15 mm. wide at the widest part. At fertilization it is broadest just below the archegonia; at maturity it is broadest at the basal end (compare fig. 48 with fig. 4 in the earlier paper [3b]).

After the embryo has differentiated its organs and has begun its final stage of development its cells become packed with food materials (fig. 45). The smaller round grains shown in the figure are starch. The proteids occur in very large subspherical masses. Not infrequently the large globule includes a smaller one. The inclusions are also sometimes angular and probably crystalloids. The latter are smaller than the globular ones. Peculiar dumb-bell-shaped bodies are also found included in the large proteid masses. Often one end is included, while the other projects freely from the surface. The nuclei of these cells often become very large and sometimes flattened. Two conspicuous nuclei of this sort are shown along the lower side of fig. 45.

The growth of the gametophyte does not destroy the nucellus, as usually happens among the gymnosperms. On the contrary, it continues to develop *pari passu* and forms an integral part of the mature seed coat. In fig. 48 it can easily be distinguished as a

separate layer of the seed coat, especially on the right side of the figure. Its tissues become lignified in precisely the same manner as those of the integument and scale. Fig. 47 shows a section through the developing testa at about the time it begins to become woody enough to be unsuitable for cutting in paraffin. The outer layer consists of a conspicuous epidermis filled with mucilaginous contents. Beneath this there is an irregular layer of cells with darkly staining contents, probably largely tannins. On the inner border next the nucellus there is a less conspicuous epidermis underlaid by several layers of elongated, thin-walled cells with very scanty contents. The larger part of the testa consists of the irregular cells shown in the central part of the figure. These cells become elongated and more tangled as the seed grows larger. At first their walls are very woody and tough, but not at all brittle. In the adult seed they turn brown, become much more brittle, and when dry are capable of being in part reduced to fine brown powder by crushing. The changes in the integument and nucellus are of the same kind as those occurring in the scale itself. The result is that in the mature seed all these parts have developed into a homogeneous structure, and ovule and scale have united to produce the seed. It resembles what might be expected to develop from a naked anatropous ovule.

Discussion

Araucaria and *Agathis* resemble one another very closely, differing only in minor points. They present a number of sharp contrasts to most other conifers. Pollination of the ovuliferous scale, very long and extensively branching pollen tubes, extruding nucelli, precocious division of the body cell, large actively motile male cells, and concentric proembryos will serve to recall some of these points of difference. Excepting *Saxegothaea*, with its protruding nucellus near which the pollen germinates, these features are very different indeed from the corresponding ones in the other families of Coniferales.

These resemblances to *Saxegothaea* have attracted the attention of a number of botanists (**16, 22, 24, 25a, 26**). Taken in connection with other resemblances they are sufficient to create a strong

probability of a real relationship between the araucarians and podocarps.

Though it has been generally recognized by botanists that the protruding nucellus is correlated with the method of pollination and extensive growth of the pollen tube, it does not appear to me that this very peculiar situation has received anything like the attention that it deserves. I have elsewhere (**3a, 3b**) expressed the opinion that pollination of the scale, coupled with an extruded nucellus, is more likely to indicate the retention of an ancient habit than the acquisition of a new one.

It must be admitted that we know comparatively little about the structures and affinities of paleozoic seeds and pollination devices. In the absence of present knowledge we must resort to more or less probable conjectures in our attempt to relate the already known facts. We do know enough, however, to make it very probable that the earliest known gymnospermous seeds are very far from being representative of the beginnings of the seed habit. They had already acquired numerous complexities. It is scarcely credible that the actual first seeds should have been provided with a deep and narrow micropyle, with devices to draw the pollen grains down into it and on into a chamber specially prepared for their reception by the breaking down of the cells of the nucellus. It is further to be noted that seeds of this type have in their pollen chambers pollen grains that show no signs of having possessed pollen tubes. It seems evident that this complexity of devices must have had a more or less extended history, and that to understand it we must try to conjecture the conditions and structures that would have been likely to be developed as intermediate stages between heterosporous pteridophytes and these paleozoic gymnosperms.

It is not alone that we do not know the history of the seed structures of these early gymnosperms that makes the problem difficult. The difficulties of relating the structures known in more modern plants to these ancient ones is no less difficult.

An analysis of the known facts will show that there are four distinct methods of accomplishing pollination and fertilization now known among gymnosperms: (1) the Cordaitales and Cycadofilicales

have a pollen chamber in the nucellus in which the pollen grains lodged; no pollen tubes are known and the indications are that they were not developed; (2) in Cycadales the pollen lodges in an already prepared pollen chamber in the nucellus and forms haustorial branching pollen tubes, which do not penetrate toward the female gametophyte and take no part in transferring the ciliated sperms to the archegonia; they are haustorial and nutritive in function; the way for the sperms is cleared by the gradual dissolution of the cells forming the bottom of the pollen chamber; (3) in most of the Coniferales the pollen passes down to the tip of the nucellus, where it puts out a pollen tube that is both nutritive and a sperm carrier; (4) the Araucarineae, and to a less extent *Saxegothaea*, are pollinated on the ovuliferous scale at a distance from the ovule, from which point a pollen tube grows toward the micropylar end of the ovule and there enters the protruding nucellus.

Another fact that seems to me especially significant in any attempt to account for the origin of these various habits is that in *Araucaria*, some podocarps (13) related to them probably, and in cycads, the embryo is not mature when the seeds are shed and keeps on growing after the seeds fall. It appears to me that this is the sort of habit one would theoretically expect to find in primitive seeds for reasons stated below. It adds strength to this supposition that Cycadales are universally recognized to be primitive plants, and that many investigators believe the araucarians to be the modern representatives, little changed in many ways, of a very ancient line and to be closely connected with the podocarps. Perhaps it will be worth while to attempt a brief analysis of the possible origins of the four classes of pollination devices mentioned above.

CYCADOFILICALES.—Whether the Cycadofilicales are more primitive than the Cordaitales is a debatable question, but that they exhibit their seeds on less modified foliar organs affords some reason for thinking that the seeds themselves are also less modified. *Physostoma elegans* (17) will serve as a starting-point in an attempt to work back to the origin of seeds of this type. In the seeds of this type the integument is split up into more or less divergent lobes

which do not closely invest the nucellus. The female gametophyte is covered by a very thin layer of nucellar tissue above. The pollen chamber occupies almost all of the exposed portion of the nucellus and probably laid bare the gametophyte at its maturity so that the free-swimming (probably) sperms had direct access to the archegonia.

One may suppose that when pollination first began the nucellus was freely exposed, and that the integument was either wanting or less developed than in *Physostoma*. Since these seeds were freely exposed on leaflike organs, there must have been developed, as the first necessary step to pollination, a sticky secretion on the nucellus to catch the microspores or pollen grains. It must be further supposed that the pollen grain was able to secure sufficient food from this secretion to maintain itself for such a length of time as was necessary for its further development, and until the gametophyte had broken through the nucellus and exposed the matured archegonia. It is supposable that the processes that produced the sticky secretion might in the course of time develop the habit of further destroying the cells of the tip of the nucellus to produce a rudimentary pollen chamber. The further step of eroding this chamber deep enough to allow access to the archegonia without waiting for the growing female gametophyte to rupture the nucellus would appear to be easy and logical.

During the development of the pollen chamber the integuments would be developing in the direction of greater efficiency in securing the deposition of the pollen on the tip of the nucellus. As they closed up the sticky secretion would be exuded as a pollination drop to catch the pollen. If the ovules stood upright gravity would effect the delivery of the pollen to the pollen chamber. In any case, the pollen would probably be retracted along with the pollen drop when it began to dry up. It is in this stage of development that the seeds of Cycadofilicales are found fossil. The reason (it appears to me) is that seeds that had been fertilized (or were far enough along to be fertilized soon) fell to the ground and continued their growth. If this were the case one would expect to find fossil only those seeds that had fallen too soon to be able to continue growth.

CYCADALES.—It appears that the cycads, ancient and modern, are closely related to Cycadofilicales. This relationship is not contradicted by the pollination devices, for they are so similar as to afford little difficulty in bridging over the gap. The method of deposition, as well as the presence of a pollen chamber in the nucellus, are as near alike as one could well expect. The greatest difference is the presence of a pollen tube in modern cycads. It has already been pointed out that this tube grows away from the female gametophyte and is exclusively haustorial in function. The pollen chamber itself provides access to the archegonia in just the way that we have conjectured for the preceding group. The real difficulty arises in supplying a convincing reason for the origin of a tube at all. If we are to homologize the pollen tube of cycads with the rhizoid of the germinating spore of their pteridophyte ancestors, it means that an organ that had been completely lost has been revived. Admitting the possibility, about which I am very dubious, of the revival of this ancient structure after the lapse of geologic ages, it is evident that it would be useful, subject to the laws of selection, and likely to be preserved. If pollination preceded fertilization only a short time in the earlier seeds, and the remaining processes took place on the ground, it is evident that what these ancient plants had attained was not the "seed habit," in the sense that we employ the term with reference to modern plants. They had merely attained the ovule and pollination habit. A real seed could be developed only if the seed structure were retained on the plant until its maturity (except the growth of the embryo itself). An advantage would certainly lie in early pollination of the ovule that would permit the further growth of the ovule even while the gametophytes were maturing. That this habit of pollination long before fertilization is an advantage is indicated by the fact that all modern seeds practice it, although it is difficult to imagine that the first seeds or ovules that were pollinated furnished food and protection on the exposed nucellus sufficient to maintain the male gametophyte for a year or more, as is commonly the case in modern conifers.

CORDAITALES.—The Cordaitales differ from the Cycadofilicales, among other things, in that their seeds occur in cones and not on

exposed foliar organs. It is an interesting and I think a significant question whether the pollination habit or the cones were developed first in this group. If we assume provisionally that the cone habit did not develop until after pollination was a fixed habit, the explanation of the origin of the latter given above might be applied to this group also. The cone habit would then have been acquired while the integuments and pollination apparatus were being perfected. The difficulties of this explanation seem to me not to lie in its application to the Cordaitales themselves, but in the assumptions that must be made in deriving the Coniferales, particularly the Araucarineae, from them.

If it be supposed that the Cordaitales as a class had all reached essentially the same stage of development of the pollination devices, and that it was comparable to that already described for the Cycadofilicales, we may then seek to see just what changes must have taken place during the evolution of modern conifers. *Ginkgo* presents almost the same devices as the cycads, and we may therefore confidently assume that an explanation that will suffice for the one will prove adequate for the other.

CONIFERALES.—Excepting for the moment the araucarians and *Saxegothaea*, the modern conifers are characterized by the pollen being caught in a pollination drop and drawn down upon the tip of the nucellus or at least into the micropyle, where it germinates. The pollen tube that is produced is both haustorial and spermiferous. It grows more or less directly down through the nucellus and delivers the male cells in the neighborhood of the archegonia. It must be noted that it thus differs very sharply from the pollen tube of the cycads and *Ginkgo*, where the pollen tube is strictly haustorial and is never even entered by the body cell or its products. It is evident therefore that *either this pollen tube is one that has altered its function and completely changed its method and direction of growth or it is a different kind of pollen tube.*

As there were no pollen tubes (probably) in the Cordaitales there is no compulsion to assume that their descendants necessarily developed a tube that behaved in the manner of the cycads. *Ginkgo*, of course, would be an exception to this statement, but

may be left out of present consideration because there is little evidence that it lies in the line of direct descent to the conifers. Since the method employed in the cycads is an entirely successful one (more so than that of the conifers, in fact), there would appear to be no reason why variations from it in a direction that would be not only of no use to the plant but a positive hindrance to it would be selected and preserved, even if they should occur. It seems very unlikely, therefore, that such a change of function did occur.

If we start with the condition actually found among the Cordaitales, where pollen grains without any tubes were deposited in a pollen chamber in the nucellus, can we see any sufficient reason for the giving up of the pollen chamber and the development of the tube? One type of ovule is just as easy to pollinate as the other, for if pollen can be gotten to the nucellar tip, there would appear to be no difficulty in getting it into the pollen chamber. If it reached the pollen chamber safely and the pollen chamber broke through so as to give the swimming sperms direct access to the archegonia, it is difficult to see what would be gained by the giving up of the chamber and the formation of a pollen tube. That the conifer method is in fact inferior and would be selected against is strongly indicated by the fact that the proportional number of good seeds in their cones is decidedly less than that of cycad cones. The evidence would thus appear to be against such a derivation of the coniferous pollen tube.

If it is difficult to see any adequate reason for the evolution of the ordinary coniferous pollen tube from the conditions found in the Cordaitales, it is vastly more difficult to imagine any adequate reason for its further evolution into the araucarian tube. We must imagine not only that the pollen chamber has been given up but that the place of pollen deposition has gradually retreated out through the micropyle and back along the scale from bad to worse. JEFFREY and CHRYSLER (11) would have us believe, not only that it did actually do this, but that to compensate itself for the disadvantage it was compelled to form extensive lateral haustorial branches and to "proliferate" the two "primitive" prothallial cells. I have not yet seen any reason advanced why the nucellus, having given up the habit of forming a pollen chamber, should have

undertaken to follow up the pollen grains by protruding itself through the micropyle. A theory beset with such manifest difficulties can be accepted only if no more probable one can be found.

ARAUCARINEAE.—The araucarians have been thought by some authors to be derived from the lycopods (**20, 4a, 4b, 22a, 22b**), and by many others to be derived directly from the Cordaitales. We have seen that the pollen tube structures do not lend any support to the derivation of the araucarians from the cordaiteans *through the other families of conifers*. Whether the pollen tube structures could be derived directly is a question that we can best attack after considering the bearing of these structures on the theory of a lycopod origin.

Miadesmia (**19**) and *Lepidocarpon* (**19**), two seed-bearing lycopods, seem to me to present the most suggestive analogies of the manner in which such a seed as that of *Araucaria* may have been evolved. I do not mean to imply that these analogies are sufficient or adequate evidence for deriving the araucarians alone or conifers as a whole from the lycopods, but merely that the araucarian pollination apparatus could be easily derived from such seeds as these plants possessed, whether they belonged to lycopods, cordaiteans, or what not.

The seeds of *Lepidocarpon* were formed in cones and not exposed as in the Cycadofilicales. The same is true of *Miadesmia*. I am inclined to attribute to this fact considerable importance. Seeds that originated on a naked foliar structure would necessarily have to be pollinated on the ovule to have any chance of success at all under any ordinary conditions of plant growth. Otherwise, the ciliated sperms would have encountered almost insuperable difficulties in reaching the archegonia and would have been limited to wet weather. It seems from such considerations that Cycadofilicales and their allies have been from the first pollinated on the nucellus, but no such compulsion rests on plants which had acquired the cone habit first. The natural, easy, and probable place for the lodgment of the earliest pollen would be between the scales anywhere. There would be far less danger of the pollen blowing away before it could become effective because of its protected

situation and far greater probability of frequently finding sufficient moisture for the swimming sperms. In fact, neither of these lycopod seeds shows any signs whatever that pollen ever lodged on the nucellus. *Miadesmia* actually possesses integumentary outgrowths that appear to be designed to prevent pollen from entering the micropyle. Though these hairs would probably keep pollen from entering the micropyle, they would serve equally well to catch it and retain it near the ovule. Sperms freed here would be in a favorable position to reach the archegonia with a minimum amount of moisture, which might very well be exuded by the cone scales, just as it is today in *Araucaria*. The reasons for the *formation* of pollen tubes in this type of pollination are no greater than in the previous type, but once formed and endowed with the habit of growth toward the archegonia, they would add immensely to the probability of fertilization, and so would tend to be selected and preserved in the evolution of the seed. Such tubes would probably always have grown toward the micropyle of the ovule because of the greater opportunity of securing suitable food in that direction. They would probably branch for the same reason that fungus hyphae branch (whatever that reason may be). Probably the main branch did not at first regularly reach the nucellus, but only came to do so later, after the nucellus had acquired the habit of secreting some chemotropically active substance. Then if the pollen tubes in search of food ever came to penetrate the nucellus before it had been broken through by the female gametophyte, they would furnish a more direct and easy route for the swimming sperms to the archegonia than for them to be freed outside the ovule as in the earlier stage. An advantageous habit of this sort would be likely to be preserved. We thus attain the state of affairs illustrated by the araucarians.

It is perhaps worth while pointing out that, in the above argument, *the various changes are not supposed to have occurred because they would be advantageous, but having occurred fortuitously to have been preserved because they were advantageous*. In contrast, the theory outlined above (11) of the derivation of pollen tubes among the conifers and araucarians requires *the derivation of disadvantageous changes and their selection and preservation notwithstanding.*

Moreover, it requires the further derivation of other structures (proliferation of the prothallial cells) to compensate for the disadvantages.

It is comparatively easy to derive the pollination apparatus of the ordinary conifers from the araucarian type by reduction, for it can be shown that each step would be an advantage, and so likely to be retained whenever it chanced to occur. In the first place, any change that would bring the pollen grains nearer the micropylar end of the ovule would shorten the distance to be traveled and so be an advantage. *Agathis* shows such a change, and there are abundant reasons for thinking it less primitive in other respects than *Araucaria*. *Saxegothaea* shows a still further stage of reduction, and there are also good reasons of other sorts for believing that it too is related to the araucarians and derived from them. The podocarps and pines illustrate the final stage where the pollen tube forming grains reach the inclosed nucellar tip before germinating. Once the pollen was deposited directly on the nucellus, changes tending to cover the nucellus by the integument and to draw the pollen down the micropyle by means of a pollination drop would be further advantages in the way of further protection to the germination tubes from drying, as well as some advantage in closing up the micropylar orifice in the maturity of the seed.

As we have seen that it would be easy to derive the Pinaceae from the araucarians so far as the seed and pollination habits are concerned, and next to impossible to reverse the order, we may now inquire whether it is possible to derive the araucarians from the Cordaitales directly in respect to the same structures. There are abundant evidences that among paleozoic gymnosperms of both great groups the nucellus either protruded from the nucellus or projected far into it. So far as I have been able to find from the literature available to me they all show pollen chambers. I suspect that this preponderance of evidence in the published accounts is due in part at least to the general opinion that pollen chambers are primitive, and so this feature has been exploited. It is conceivable, at any rate, that some of the paleozoic gymnosperms did not have pollen chambers and were not pollinated on the nucellus, but on the

scale. If such evidence should be forthcoming, the line of argument that has been used in connection with the lycopod seeds could be equally applied to the Cordaitales. The cordaitean seeds were formed in cones, and I should strongly expect that some of them were pollinated on the scale instead of the nucellus.

The theory would run something like this. When the seed habit was developed, the plants were in the midst of acquiring the cone habit. Pollination would therefore differ in nearly related plants. The ones that first perfected the pollination habit would be likely from the first to be pollinated on the nucellus. The ones forming cones first probably acquired thereby the habit of pollinating the scale. Some of these may have deposited the pollen so near the nucellus that they soon passed through the intervening stages and so show no special differences from those that always had had the pollen on the nucellus. The history of the pollen chamber would be the same as that already outlined for the Cycadofilicales. Whether these ancient plants that gave rise to modern conifers were more like araucarians or other modern conifers in other respects cannot, of course, be decided on these grounds. It does seem to me that the mesozoic conifers very probably did resemble the araucarians in respect to the seed and pollination habit. This might be equally true whether they resembled the araucarians in their vegetative structures or were more like the Abietineae, as has been vigorously maintained in recent years by some anatomists (10).

The theory of the pollen tube above outlined is applicable to the structure of the male gametophyte itself. There is nothing to be explained away, as must be done if we attempt to derive the araucarian type from the pine type (11). The numerous prothallial cells are not then to be thought of as something to be explained away, but as what is left of the ancient prothallus. A figure (2, fig. 2) from Miss BENSON'S paper on *Lagenostoma* seems to me to be capable of another interpretation than the one given by the author. The figure shows a number of pollen grains in the pollen chamber. At the upper right of the figure is a group of one large and several small cells. The large cell is labeled "a sperm," and the smaller ones are said to be probably fungal

cells. I should like to suggest that they strongly resemble a group of prothallial cells about a body cell or sperm as they appear in *Araucaria*. So far as it goes, it seems to me, the evidence is that there were prothallial cells in the paleozoic pollen grains, notwithstanding that eminent botanists have interpreted the evidence to the contrary (**7**).

I have elsewhere (**3a**) called attention to the very large male cells of *Araucaria*, and EAMES has recently (**8**) shown that they are present in *Agathis* also. A further peculiarity in their formation is exhibited by *Araucaria brasiliensis*, in that the division of the body cells occurs a long time before fertilization and not about simultaneously (**7**) with the division of the central cell a few days before fertilization. This division usually occurs outside of the nucellus a month or even two months before the pollen tube has actually reached the archegonia. Not only are the male cells long-lived, but they appear to be more active and independent than those of most conifers. This appears to me to be a primitive and unspecialized behavior, and one that would be unlikely to be derived secondarily from the condition now obtaining in the pines.

The male cells of *Araucaria* pass through the neck of the archegonium without injuring it. COULTER and CHAMBERLAIN (**7**) assert that the pollen tube of the Pinaceae destroys the neck, though LAWSON (**12c**) has recorded that the pollen tube of *Sciadopitys* passes between the neck cells. Among the Taxaceae the neck cells are sometimes destroyed (*Torreya*, COULTER and LAND **6**), and sometimes the male cells pass through without injuring them (*Phyllocladus*, Miss YOUNG **26**), just as in *Araucaria*. In *Agathis* (**8**) the male cells enter the top of the archegonium to one side of the neck cells, which are thereby broken loose from their anchorage to the jacket. In *Podocarpus* the necks appear to be broken through by the neck, though SINNOTT'S (**21**) statement is not specific as to whether the neck cells are destroyed or not. In *Cephalotaxus* (**12b**) the neck cells are probably destroyed by the entrance of the tube between them. In *Cryptomeria*, which externally resembles some species of *Araucaria* very closely and has other suggestions of affinity as well, the male cells are said (**12a**) to pass between the neck cells, but to injure them in doing

so. *Araucaria* finds again its closest resemblances among the Taxaceae. The habit of entering the archegonium between the neck cells without injuring them is an old one, dating back to Archegoniatae generally. The habit in the araucarians of liberating the male cells outside of the archegonium and allowing them to enter it under their own power of movement is doubtless more primitive than that prevailing among the Pinaceae, where the tube actually delivers the male cells inside the archegonium in many cases.

The female gametophyte is very similar to that of most conifers. Attention has been called to the apparently peculiar method of wall formation in the transformation of the free-nuclear state to the walled prothallus. Since that was written (**3b**), SAXTON has published an account of the life history of *Tetraclinis articulata* (**18b**), in which he shows a photograph (fig. 6 of his paper) in which three of the nuclei occupy a position in what is elsewhere supposed to be the wall of the forming alveoli. Possibly this may indicate that walls are formed in this plant in the same way as in *Araucaria*.

The multinucleate condition of the prothallus at fertilization time is not peculiar to *Araucaria*, being now recorded in several other genera (*Agathis*, *Cryptomeria*, etc.). It is probably more widespread than the literature indicates at present.

The late stage at which the ventral canal nucleus is cut off, and the lack of any trace of a wall are certainly not evidences of primitive behavior. In fact, there are very few evidences that the female gametophyte has lagged in its development behind conifers in general. That is, it seems to me, as it should be. The male gametophyte has retained a lot of primitive characters, because they are associated with the habit of pollinating the scale. These influences do not affect the female gametophyte, and it has therefore gone on in the course of evolution much as other conifers have done.

The persistence of the male cytoplasm in the egg has now been recorded for a number of genera of the Araucarineae, Podocarpineae, Taxodineae, and Cupressineae, but I have seen no record of it among the Abietineae. The majority of these records relate to genera (*Agathis*, *Phyllocladus*, *Podocarpus*, *Torreya*, and *Cephalo-*

taxus) that have suggestive resemblances in other respects to one another.

EAMES (8) has laid special stress on the fact that in *Agathis* the fusion nucleus remains in the center of the archegonium and that its divisions are limited to a restricted part of the egg cytoplasm. He looks on this as a specialization. I should be inclined to minimize the importance of this feature, for in *Araucaria* division occurs wherever the impact of the male cells has left the egg nucleus. Neither is the restriction a noticeable feature further than is determined by the fact that the proembryonic free nuclei are restricted to the limits of the male cytoplasm that envelops them.

The irregular division in the proembryo, the indefinite number of nuclei formed, and the method of their arrangement distinguish *Araucaria* rather sharply from the Abietineae, though some or all of these features are paralleled among the other tribes.

The number of cells in the proembryo before elongation, and the time of wall formation vary widely. Abietineae usually, at least, have four tiers of four cells each. In the Cupressineae the cells are usually fewer and not so regularly arranged. Among the Taxaceae the numbers run much higher (18–32) and the arrangement is still less regular. Walls form somewhere about the 8-celled stage in Pinaceae, but at widely different stages among the Taxaceae.

In all other recorded conifers, excepting *Actinostrobus* (**18a**), the proembryo is arranged in more or less regular vertical tiers and the growth is downward. In the araucarians the embryo is not tiered, but concentric at the time walls are formed. It takes on a pseudo-tiered appearance later through the elongation of the upper cells of the concentric outer layer to form the suspensor and the lower ones to form a cap. In this respect the proembyro is unique, though *Cephalotaxus* (**12b, 23**), *Sciadopitys* (**17**), and some species of *Podocarpus* (**21**) resemble it in having a cap below the embryo cells. In neither of these, however, is the embryo group completely surrounded as in the araucarians. These resemblances do not contradict a relationship between araucarians and taxads, nor do they add very much strength to the evidence for it. The structure of the proembryo is so different from that of the Abietineae that it is not easy to see how it could have been developed from it.

Notwithstanding the opinions (8, 23) expressed by other authors as to the specialization of the proembryo of the araucarians, I am inclined to think that too much stress has been placed on the appearance it presents after elongation has begun. At that time the very definite cap, the suspensors, and the inclosed embryo group give it an appearance of specialization that is not representative of its method of development. As I have pointed out in a preceding paragraph, this very definite structure arises from a group of free nuclei that do nothing in a definite and regular fashion. The number of nuclei is indefinite, the arrangement is that of an irregular mass, the order of wall formation varies, in short nothing is definite or fixed except that the upper and lower nuclei of the mass will elongate and result in the production of a proembryo in which the position and function of the various cells appear to have been planned with the greatest care. The course of development is, in fact, far less regular and definite than that of the Abietineae, though the result is far more striking.

The formation of a secondary suspensor region from the base of the mass of cells developed from the embryo group is a feature that has not been recorded for other conifers, so far as I have been able to discover from the literature available. The nearest approach to it is in *Torreya taxifolia* (6), another taxad, where there is said to be a wave of elongation beginning with the second tier and involving the successive tiers downward until finally cells formed from the embryo groups are involved. It is not unlikely that this feature may be found less rare than the records at present indicate, for our knowledge of the later development of the embryo is still very meager in conifers generally.

Conclusions

1. The structure and development of the pollen tube, processes of fertilization, and the structure and development of the embryo are such that it seems extremely improbable that they could have been derived from the analogous structures as represented in modern Abietineae.

2. The structure of the seed and pollination apparatus of the araucarians could be readily derived from the type of seeds or ovules represented by such lycopods as *Miadesmia*.

3. There is some reason to suppose that some of the Cordaitales may have had ovules of the same general type as the lycopods just mentioned. If so, they were probably pollinated on the scale and might have given rise to modern conifers.

4. It would be possible to derive modern conifers from a mesozoic stock which had ovules and pollination apparatus comparable to that now possessed by the araucarians.

Summary

1. Pollination occurs on the scale at a distance from the nucellus.

2. The pollen tube is very long and gives rise to many small lateral haustorial branches. It combines features of conifers and cycads to a certain extent.

3. Reasons are adduced to show that this is probably an extremely primitive form of tube, having come down from very remote times little changed.

4. The body cell divides in the extra-nucellar part of the tube a month or more before fertilization. The central cells of the archegonium divides very late or perhaps not at all, except in cases of delayed fertilization.

5. The male cells are very large and unusually active, as well as long-lived.

6. Blepharoplast-like bodies are found in the male cytoplasm.

7. The male cells pass through the neck without injuring the cells.

8. The male cell comes into violent contact with the egg and frequently displaces it.

9. The free nuclear divisions of the proembryo are restricted to the male cytoplasm that surrounds the fusion nucleus, which persists and grows with the proembryo.

10. The male cytoplasm around the older proembryo may be surrounded by a membrane.

11. The number of free nuclei in the embryo varies from 32 to 45 or perhaps more.

12. When walls form the free nuclei are arranged concentrically.

13. The upper peripheral nuclei form the suspensor, the lower ones the cap, and the middle girdle elongates to unite cap and suspensor.

14. The central cells of the proembryo alone take part in forming the embryo.

15. In the growth of the embryonic group the cap is thrust aside and a cylinder of meristematic tissue is organized.

16. The upper portion of the embryonic cylinder functions as a secondary suspensor.

17. The definitive embryo is organized out of a portion of the cells arising from the development of the embryo group of the proembryo.

18. It is dicotyledonous, has resin ducts in the cortex but not in the wood, and is stored full of food materials (large proteid granules and smaller starch grains).

19. The cells of the prothallus become very large and crowded with food.

20. The nucellus persists and becomes a part of the testa of the seed.

21. The embryo continues intraseminal growth after the seeds are shed.

22. It is concluded that, so far as the pollination apparatus and seed structure are concerned, the Araucarineae could be derived from the lycopods, or perhaps from the Cordaitales, but not from the Abietineae. The latter might be derived from a primitive mesozoic stock resembling the araucarians in respect to these features.

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EXPLANATION OF PLATES I–III

FIG. 1.—A pollen tube branching after entering the nucellus; the tip of the tube is just at the upper border of the figure.

FIG. 2.—A part of the body cell, showing part of the upper male nucleus and what appears to be the remains of the nuclear spindle concerned in the division of the body cell nucleus; the male nuclei are in the resting state, but the cytoplasm has not yet begun to divide.

FIG. 3.—The two male nuclei in a body cell whose cytoplasm has not yet completely separated into distinct male cells; ×250.

FIG. 4.—Two male nuclei in a pollen tube running horizontally between the nucellar cap and the female gametophyte; the cytoplasm is unusually scanty and shows no sign of division, nor does either nucleus appear larger or more active than the other; ×250.

FIG. 5.—A binucleate body cell rounding a sharp corner of the pollen tube where it turns back from the surface of the scale to enter the nucellus; a part of the pollen tube wall is shown above and another part in the bend on the right; ×250.

FIG. 6.—A fully formed male cell or sperm about half-way down the pollen tube; the nucleus is at the forward end and a fragment of the degenerating nucleus that should have formed a separate male cell out of the part of the cytoplasm above and to the left of the cleavage furrow; ×250.

FIG. 7.—The egg nucleus and disintegrating ventral canal nucleus; ×250.

FIG. 8.—Upper end of an archegonium with a large ventral canal nucleus (at the top), a small egg nucleus (bottom), and a small extra nucleus between; ×250.

FIG. 9.—A mature egg nucleus showing the dense chromatin masses distributed on the delicate linin network and to a less extent in contact with the nuclear membrane; the wrinkles in the nuclear membrane are doubtless due to the effects of the reagents used in preparation; ×560.

FIG. 10.—The neck of an archegonium through which a pair of male cells has entered; note that the neck has not been ruptured, though the passage is

very much smaller than the diameter of a male cell; the figure also shows how the egg cytoplasm has been crowded out through the neck by the entrance of the male cells; ×250.

FIG. 11.—Fertilization: the male nucleus is above; both nuclei are enveloped already in the male cytoplasm, which is distinguished from the egg cytoplasm around it by being much denser; this figure shows the only case observed in which the male nucleus is larger than the female; ×250.

FIGS. 12 and 13.—Two consecutive sections through a male and female nucleus in the act of fusing; the male nucleus is to the left of the figure; the nuclear membrane has broken down in the middle region of contact (fig. 13), but not throughout (fig. 12); each nucleus is enveloped in a distinct sheath of cytoplasm, probably derived from the kinoplasmic layer sometimes surrounding the egg nucleus, as well as from the male cytoplasm; ×250.

FIG. 14.—A median section through two nuclei in which the nuclear membrane had not yet broken down, showing how the male nucleus flattens out and applies itself to the curved surface of the egg nucleus; ×250.

FIG. 15.—Fusion of two nuclei showing the fine-grained nuclear contents and the weakening of the nuclear membranes; two blepharoplast-like bodies are also shown; the left-hand one may be, possibly, a vegetative nucleus in an advanced stage of degeneration; ×250.

FIG. 16.—The second male cell in an archegonium: the male cell is cut in the median plane, but only one of the two nuclei of the proembryo below is shown; the dark portion in the center is the nucleus crowded full of large masses of chromatin-like material; around it is seen the zone of male cytoplasm appearing lighter than the surrounding egg cytoplasm or the inclosed nucleus; this cell has probably become considerably changed through degeneration; ×250.

FIG. 17.—Another section through the same archegonium as the preceding, showing one of the blepharoplast-like bodies in the edge of the male cytoplasm and a nearly median section of the 2-celled proembryo; ×250.

FIG. 18.—Median section of a 2-celled proembryo; ×250.

FIG. 19.—Another 2-celled proembryo; ×250; figs. 17–19 show that the first division may occur in any plane, horizontal, vertical, or oblique.

FIG. 20.—Median section of a 4-nucleate proembryo; ×250.

FIG. 21.—A 6-nucleate proembryo; ×250.

FIG. 22.—A 5-nucleate proembryo; ×250.

FIG. 23.—A 4-nucleate proembryo with all the nuclei in nearly the same vertical plane and at the bottom of the cytoplasm; ×250.

FIG. 24.—A 9-nucleate proembryo; ×250.

FIGS. 25–29.—Proembryos with 15–40 nuclei variously arranged, but none with exactly 16 or 32; ×250.

FIG. 30.—A 45-nucleate proembryo, with the nuclei properly arranged for wall formation; the cap nuclei are already beginning to elongate; ×250.

FIG. 31.—The formation of walls and elongation of suspensors before the cap cells have begun to elongate or have completed wall formation; ×250.

FIG. 32.—Wall formation and elongation in the cap cells before either elongation or wall formation has begun in the suspensors; a small part of the membrane that sometimes forms above or even around the proembryo is shown at the upper right of the figure; ×250.

FIGS. 33 and 34.—A proembryo cut full length, showing all its parts after the elongation of the suspensors has begun; fig. 33 shows the expanded top of the suspensors crowded up against the neck of the archegonium; the archegonium jacket membrane is much stretched but has not broken nor has the neck been ruptured; fig. 34 shows the suspensors and the progress of destruction of the cells of the gametophyte; ×62.

FIG. 35.—Tip of a proembryo, showing the bottom of the suspensors, the group of embryo-forming cells, and the cap; note that the embryo is not properly a tiered one, and that the cell contents of the three regions are exceedingly similar; the walls of the cap cells are also seen not to be specially thicker or otherwise prepared for mechanical penetration; ×250.

FIG. 36.—Three proembryos in competition for the favored position in the endosperm; the multinucleate condition of some of the endosperm cells is also shown; the fine grains in these cells are starch and the large light colored patches are vacuoles; ×62.

FIG. 37.—The struggle for supremacy during which the proembryos coil around one another and greatly erode the gametophyte; ×20.

FIG. 38.—The beginnings of growth in the embryonic group of cells; the suspensor cells lose their cytoplasm and become distended, and the cap cells shrink and degenerate; ×175.

FIG. 39.—Further growth in the embryo group: the cap has been crushed and is being thrust to one side; cell division is more rapid in the tip region of the future embryo, while the upper cells are beginning to elongate, foreshadowing the production in that region of the secondary suspensor; a few cells of the primary suspensor are shown at the top; ×250.

FIG. 40.—The secondary suspensors pushing the meristematic apex deep into the endosperm; note the massive character of the secondary suspensor when compared with the slender primary one; ×62.

FIG. 41.—A later stage when the meristematic region has become large; the differentiation of the body regions shown in the next figure will follow shortly after the stage shown in this figure; ×20.

FIG. 42.—An embryo just after all the main body regions have been differentiated; ×7.

FIG. 43.—Transverse section through the hypocotyl of a nearly mature embryo, showing the vascular ring, resin ducts, and cells crowded full of starch and proteids; ×15.

FIG. 44.—Section through the cotyledons; ×15.


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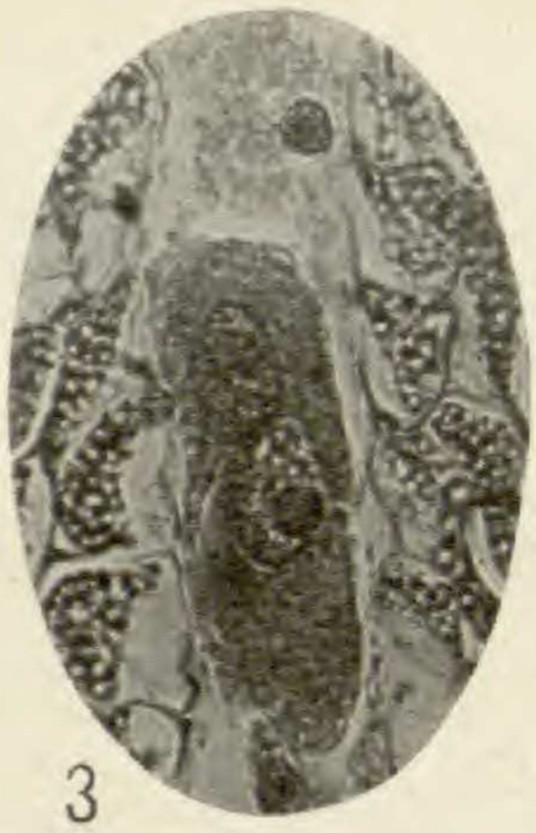
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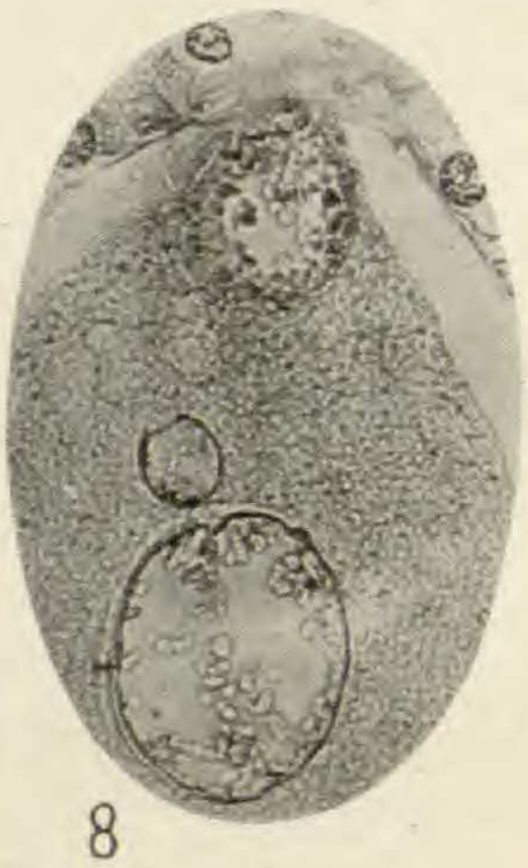
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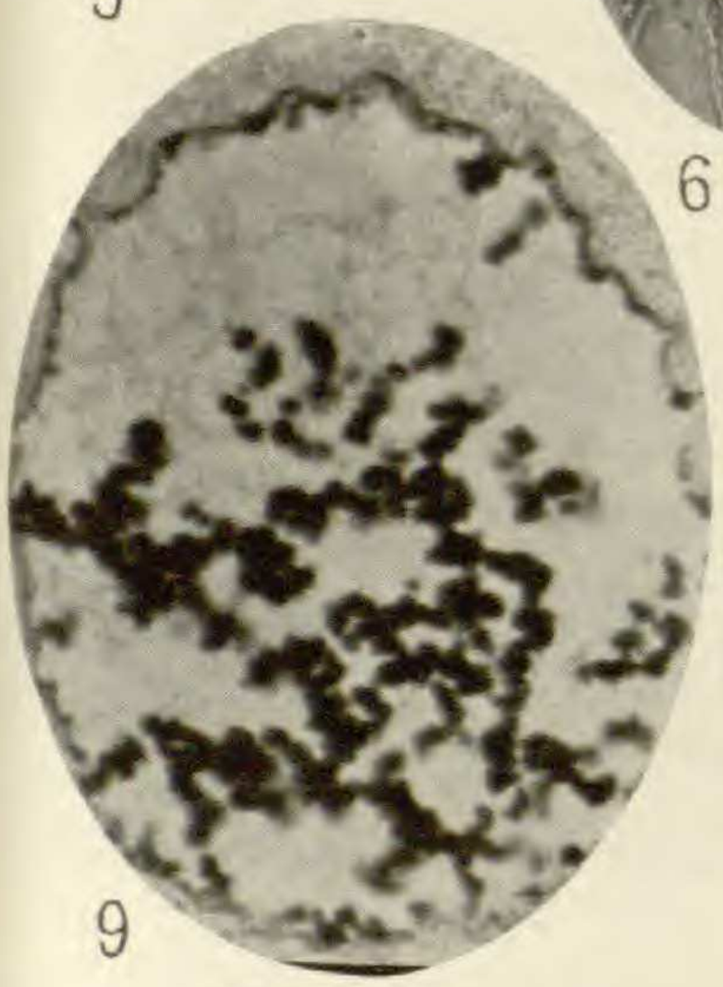
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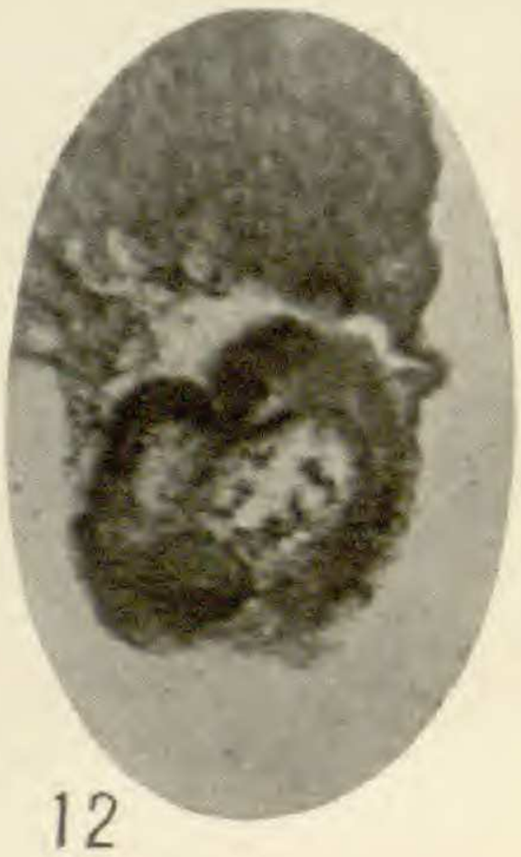
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BURLINGAME on ARAUCARIA